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Scientific and Technical Information Center

SEARCH REQUEST FORM

Requester's Full Name: MARCELA CORDERO GARCIA Examiner # : 80381 Date: 4/27/05
Art Unit: 1654 Phone Number: 2-2939 Serial Number: 10/030,230
Location (Bldg/Room#): REM3C35 (Mailbox #): 3C18 Results Format Preferred (circle): PAPER DISK
****

To ensure an efficient and quality search, please attach a copy of the cover sheet, claims, and abstract or fill out the following:

Title of Invention: METHOD FOR PURIFYING GRANULOCYTE COLONY STIMULATING FACTOR

Inventors (please provide full names): SEE BIB DATA SHEET (ATTACHED)

Earliest Priority Date: 7/8/99

Search Topic:
Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc., if known.

For Sequence Searches Only ***Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.***

PLEASE SRCH CLAIM 1

CLAIM 1

A PROCESS FOR PURIFYING GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) FROM A BIOLOGICAL SAMPLE, SAID PROCESS COMPRISING:

a) REDUCING THE VOLUME OF THE BIOLOGICAL SAMPLE CONTAINING G-CSF BY HYDROPHOBIC INTERACTION CHROMATOGRAPHY TO OBTAIN A CONCENTRATED, DESALTED AND ENRICHED FRACTION

b) PASSING THE CONCENTRATED FRACTION OVER HYDROXYAPATITE UNDER CONDITIONS WHERE G-CSF IS WEAKLY BOUND TO OBTAIN A CONCENTRATED, DESALTED, AND ENRICHED FRACTION CONTAINING G-CSF; AND

c) COLLECTING THE G-CSF

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BIBDATASHEET

CONFIRMATION NO. 9208

Bib Data Sheet

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
10/030,230	10/23/2002 RULE	424	1654	03806.0531

APPLICANTS

Jacques Dumas, Bussy Saint Martin, FRANCE;

Lucien Rey, Pyla-Sur-Mer, FRANCE;
Edoardo Sarubbi, Fontenay Sous Bois, FRANCE;

** CONTINUING DATA **********

This application is a 371 of PCT/FR00/01937 07/06/2000

** FOREIGN APPLICATIONS **********

FRANCE 99 08831 07/08/1999

Foreign Priority claimed ☐ yes ☐ no 35 USC 119 (a-d) conditions met ☐ yes ☐ no ☐ Met after Allowance Verified and Acknowledged _____ Examiner's Signature _____ Initials	STATE OR COUNTRY FRANCE	SHEETS DRAWING 7	TOTAL CLAIMS 15	INDEPENDENT CLAIMS 2

ADDRESS
22852
FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER
LLP
901 NEW YORK AVENUE, NW
WASHINGTON , DC
20001-4413

TITLE
Method for purifying granulocyte colony stimulating factor

FILING FEE RECEIVED 1020	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue)

STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 152615

TO: Marcela Cordero Garcia
Location: REM/3C35/3C18
Art Unit: 1654
Friday, May 06, 2005

Case Serial Number: 10/030230

From: Toby Port

Location: Biotech-Chem Library
REM1-A59
Phone: 272-2523

toby.port@uspto.gov

Search Notes

Dear Examiner Cordero Garcia,

Here are the results of your search.
Please feel free to contact me if you have any questions.

Toby Port

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* The CA roles and document type information have been removed from *
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* available and contains the CA role and document type information. *
*                                                                    *
**********************************************************************

Crossover limits have been increased.  See HELP CROSSOVER for details.

Experimental and calculated property data are now available. For more
information enter HELP PROP at an arrow prompt in the file or refer
to the file summary sheet on the web at:
http://www.cas.org/ONLINE/DBSS/registryss.html


L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN


=> d rn cn l1
L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2005 ACS on STN
RN   143011-72-7  REGISTRY
CN   Colony-stimulating factor, granulocyte (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   CSF-β
CN   G-CSF
CN   GM-DF
CN   Granocyte
CN   Granulocyte colony-stimulating factor
CN   MGI 2
CN   Pluripoietin


=> d que l2
L2             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  1306-06-5/RN
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=> d rn cn l2

L2   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2005 ACS on STN
RN   1306-06-5  REGISTRY
CN   Hydroxylapatite (Ca5(OH)(PO4)3) (9CI)  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Hydroxylapatite (8CI)
OTHER NAMES:
CN   Alveograf
CN   AMDRY 6021
CN   Apaceram
CN   APAFILL-G
CN   Apatite
CN   Apatite hydroxide (Ca10(PO4)6(OH)2)
CN   APP 600
CN   Boneceram P
CN   Bonfil
CN   Calcium hydroxyapatite
CN   Captal
CN   Captal 90
CN   Captal R
CN   Ceratite
CN   Durapatite
CN   FKI
CN   HA 200
CN   HA 300BP
CN   HAP
CN   HAP (mineral)
CN   HAP 08NP
CN   HAP-B
CN   HW 004
CN   HW 004 (mineral)
CN   Hy-Apatite
CN   Hydroxyapatite
CN   Hydroxypol
CN   Interpore 200
CN   Interpore 500
CN   Monite
CN   PeriApatite
CN   ProOsteon
CN   Supertite 10
CN   Synamel
CN   Tri-Tab
CN   Win 40350


=> file caplus; d que 16; d que 19; d que 111; d que 112
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FILE COVERS 1907 - 6 May 2005 VOL 142 ISS 20
FILE LAST UPDATED: 5 May 2005 (20050505/ED)

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L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L2             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  1306-06-5/RN
L3          9750 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L4         18620 SEA FILE=CAPLUS ABB=ON  PLU=ON  L2 OR HYDROXYLAPATITE+PFT/CT
                 OR APP 600 OR AMDRY 6021 OR HA 200 OR HA 300BP OR HAP OR HW
                 004 OR HAP B OR HAP 08NP
L5        173049 SEA FILE=CAPLUS ABB=ON  PLU=ON  CHROMATOGRAPHY/CW
L6             1 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3 AND L4 AND L5


L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L3          9750 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L5        173049 SEA FILE=CAPLUS ABB=ON  PLU=ON  CHROMATOGRAPHY/CW
L7            59 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3 AND L5
L8            44 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 (L) PUR/RL
L9            22 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7 AND L8


L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L3          9750 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L5        173049 SEA FILE=CAPLUS ABB=ON  PLU=ON  CHROMATOGRAPHY/CW
L7            59 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3 AND L5
L10          356 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 (L) PREP/RL
L11           24 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7 AND L10


L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L3          9750 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L5        173049 SEA FILE=CAPLUS ABB=ON  PLU=ON  CHROMATOGRAPHY/CW
L7            59 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3 AND L5
L8            44 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 (L) PUR/RL
L9            22 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7 AND L8
L10          356 SEA FILE=CAPLUS ABB=ON  PLU=ON  L1 (L) PREP/RL
L11           24 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7 AND L10
L12           24 SEA FILE=CAPLUS ABB=ON  PLU=ON  L9 OR L11
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=> file medline; d que l16; d que l22
FILE 'MEDLINE' ENTERED AT 12:11:52 ON 06 MAY 2005

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 On December 19, 2004, the 2005 MeSH terms were loaded.

 The MEDLINE reload for 2005 is now available.  For details enter HELP
 RLOAD at an arrow promt (=>).  See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html

 OLDMEDLINE now back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2005 vocabulary.

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L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L2             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  1306-06-5/RN
L13        12827 SEA FILE=MEDLINE ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L14         6783 SEA FILE=MEDLINE ABB=ON  PLU=ON  L2 OR HYDROXYLAPATITE+PFT/CT
                 OR APP 600 OR AMDRY 6021 OR HA 200 OR HA 300BP OR HAP OR HW
                 004 OR HAP B OR HAP 08NP
L15       400631 SEA FILE=MEDLINE ABB=ON  PLU=ON  CHROMATOGRAPH?
L16            1 SEA FILE=MEDLINE ABB=ON  PLU=ON  L13 AND L14 AND L15



L15       400631 SEA FILE=MEDLINE ABB=ON  PLU=ON  CHROMATOGRAPH?
L18         7584 SEA FILE=MEDLINE ABB=ON  PLU=ON  GRANULOCYTE COLONY-STIMULATING
                  FACTOR/CT
L19         4112 SEA FILE=MEDLINE ABB=ON  PLU=ON  L18/MAJ
L20           19 SEA FILE=MEDLINE ABB=ON  PLU=ON  L19 (L) IP/CT
L21           11 SEA FILE=MEDLINE ABB=ON  PLU=ON  L20 AND L15
L22            6 SEA FILE=MEDLINE ABB=ON  PLU=ON  L21 NOT (PICHIA OR STROMAL OR
                 DIMER? OR LIGAND OR GLYCOL)/TI

=> s l16 or l22
L54            7 L16 OR L22


=> file embase; d que l26; d que l32
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substance identification.

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L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L2             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  1306-06-5/RN
L23        20200 SEA FILE=EMBASE ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L24         6967 SEA FILE=EMBASE ABB=ON  PLU=ON  L2 OR HYDROXYLAPATITE+PFT/CT
                 OR APP 600 OR AMDRY 6021 OR HA 200 OR HA 300BP OR HAP OR HW
                 004 OR HAP B OR HAP 08NP
L25       299366 SEA FILE=EMBASE ABB=ON  PLU=ON  CHROMATOGRAPH?
L26            0 SEA FILE=EMBASE ABB=ON  PLU=ON  L23 AND L24 AND L25


L25       299366 SEA FILE=EMBASE ABB=ON  PLU=ON  CHROMATOGRAPH?
L28        13008 SEA FILE=EMBASE ABB=ON  PLU=ON  GRANULOCYTE COLONY STIMULATING
                 FACTOR/CT
L30         4733 SEA FILE=EMBASE ABB=ON  PLU=ON  L28/MAJ
L31           30 SEA FILE=EMBASE ABB=ON  PLU=ON  L30 AND L25
L32            8 SEA FILE=EMBASE ABB=ON  PLU=ON  L31 AND (ISOLATION OR PURIF?
                 OR RETENTION)/TI


=> file biosis; d que l36; d que 142
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CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
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RECORDS LAST ADDED: 4 May 2005 (20050504/ED)

FILE RELOADED: 19 October 2003.


L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L2             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  1306-06-5/RN
L33        15668 SEA FILE=BIOSIS ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L34         6240 SEA FILE=BIOSIS ABB=ON  PLU=ON  L2 OR HYDROXYLAPATITE+PFT/CT
                 OR APP 600 OR AMDRY 6021 OR HA 200 OR HA 300BP OR HAP OR HW
                 004 OR HAP B OR HAP 08NP
L35       384841 SEA FILE=BIOSIS ABB=ON  PLU=ON  CHROMATOG?
L36            1 SEA FILE=BIOSIS ABB=ON  PLU=ON  L33 AND L34 AND L35


L1             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  143011-72-7/RN
L33        15668 SEA FILE=BIOSIS ABB=ON  PLU=ON  L1 OR GRANUL? COLON? STIMUL?
                 FACTOR OR G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR
                 PLURIPOIETIN
L35       384841 SEA FILE=BIOSIS ABB=ON  PLU=ON  CHROMATOG?
L38      2073071 SEA FILE=BIOSIS ABB=ON  PLU=ON  ISOLAT? OR PURIF? OR CONCENT?
L41           40 SEA FILE=BIOSIS ABB=ON  PLU=ON  L33 (8A) L38 AND L35
L42           10 SEA FILE=BIOSIS ABB=ON  PLU=ON  L41 AND (PURIFICATION OR
```

```
QUANTITATION OR MICROPREP? OR ISOLATION)/TI NOT CLOVER/TI


=> s l36 or l42
L55          11 L36 OR L42

=> file wpix; d que l47; d que l49
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FILE LAST UPDATED:           4 MAY 2005      <20050504/UP>
MOST RECENT DERWENT UPDATE:     200528         <200528/DW>
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    FOR DETAILS. <<<


L43          1222 SEA FILE=WPIX ABB=ON  PLU=ON  GRANUL? COLON? STIMUL? FACTOR OR
                  G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR PLURIPOIETIN
L44          3463 SEA FILE=WPIX ABB=ON  PLU=ON  HYDROXYLAPATITE OR HYDROXYAPATITE
                   OR APP 600 OR AMDRY 6021 OR HA 200 OR HA 300BP OR HAP OR HW
                  004 OR HAP B OR HAP 08NP
L45         45669 SEA FILE=WPIX ABB=ON  PLU=ON  CHROMATOGRA?
L46             2 SEA FILE=WPIX ABB=ON  PLU=ON  L43 AND L44 AND L45
L47             1 SEA FILE=WPIX ABB=ON  PLU=ON  L46 NOT HERPES/TI



L43          1222 SEA FILE=WPIX ABB=ON  PLU=ON  GRANUL? COLON? STIMUL? FACTOR OR
                  G CSF OR GCSF OR RM DF OR GRANUCYTE OR MGI OR PLURIPOIETIN
L45         45669 SEA FILE=WPIX ABB=ON  PLU=ON  CHROMATOGRA?
L48            12 SEA FILE=WPIX ABB=ON  PLU=ON  L43 (10A) (ISOLAT? OR PURIF? OR
                  CONC?) AND L45
L49             7 SEA FILE=WPIX ABB=ON  PLU=ON  L48 NOT (HAEMATO? OR MASTITIS OR
                  SUPERGENE OR SULFATE OR MONOCLONAL)/TI


=> s l47 or l49
L56             7 L47 OR L49
```

```
=> dup rem l54 l12 l32 l55 l56
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PROCESSING COMPLETED FOR L12
PROCESSING COMPLETED FOR L32
PROCESSING COMPLETED FOR L55
PROCESSING COMPLETED FOR L56
L57           46 DUP REM L54 L12 L32 L55 L56 (11 DUPLICATES REMOVED)
                 ANSWERS '1-7' FROM FILE MEDLINE
                 ANSWERS '8-31' FROM FILE CAPLUS
                 ANSWERS '32-38' FROM FILE EMBASE
                 ANSWERS '39-43' FROM FILE BIOSIS
                 ANSWERS '44-46' FROM FILE WPIX

=> d ibib ed ab l57 1-43; d ibib ab abex l57 44-46; file home

L57  ANSWER 1 OF 46     MEDLINE on STN                    DUPLICATE 5
ACCESSION NUMBER:   2001573781     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 11680888
TITLE:              Copper/zinc superoxide dismutase is phosphorylated and
                    modulated specifically by granulocyte-
                    colony stimulating factor in
                    myeloid cells.
AUTHOR:             Csar X F; Wilson N J; Strike P; Sparrow L; McMahon K A;
                    Ward A C; Hamilton J A
CORPORATE SOURCE:   University of Melbourne, Arthritis and Inflammation
                    Research Centre, Department of Medicine, Royal Melbourne
                    Hospital, Parkville 3050, Victoria, Australia..
                    xfc@unimelb.edu.au
SOURCE:             Proteomics, (2001 Mar) 1 (3) 435-43.
                    Journal code: 101092707. ISSN: 1615-9853.
PUB. COUNTRY:       Germany: Germany, Federal Republic of
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200112
ENTRY DATE:         Entered STN: 20011030
                    Last Updated on STN: 20020123
                    Entered Medline: 20011218
ED   Entered STN: 20011030
     Last Updated on STN: 20020123
     Entered Medline: 20011218
AB   Using two-dimensional sodium dodecyl sulfate polyacrylamide gel
     electrophoresis (2-D SDS-PAGE) of 32P-labeled cytosolic and membrane
     extracts, we identified a 21.5 kDa phosphoprotein with an isoelectric
```

point of 6.0 in NFS-60 cells that was phosphorylated maximally at 15 min by treatment with **granulocyte-colony stimulating factor** (**G-CSF**) but not with interlevkin-3 (IL-3) or colony-stimulating factor-1 (macrophage-colony stimulating factor (CSF-1 (M-CSF)). The phosphorylation of this protein, designated 21.5/6.0, was unaffected by a series of antiproliferative agents [32]. These findings suggested that the 21.5/6.0 phosphoprotein may be involved in specific **G-CSF**-mediated biological responses such as activation and/or differentiation. We sought to characterize this 21.5/6.0 by a novel combination of 2-D SDS-PAGE and hydroxyapatite (HTP)-**chromatography**. Amino acid sequence determination of 21.5/6.0 revealed it to share a high level of homology with copper/zinc superoxide dismutase (Cu/Zn-SOD), indicating that a Cu/Zn-SOD is phosphorylated following treatment with **G-CSF**. This is the first report of the phosphorylation and possible involvement of Cu/Zn-SOD protein in granulocyte activation/differentiation events. In addition, Cu/Zn-SOD levels and activity were diminished by **G-CSF** but not IL-3 treatment. This new protocol combining 2-D SDS-PAGE and HTP-**chromatography** allows the characterization of low abundance phosphoproteins involved in the cellular responses to **G-CSF** and presumably to other cytokines/growth factors.

L57 ANSWER 2 OF 46 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1999394032 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10464766
TITLE: Purification and characterization of two recombinant human granulocyte colony-stimulating factor glycoforms. Pharmacokinetic and activity studies of single-dose administration in mice.
AUTHOR: Rotondaro L; De Paolis E; Ferrero D; D'Alatri L; Raucci G; Fabbri A; Gerwig G J; Kamerling J P; Mariani M F; Mele A; De Santis R
CORPORATE SOURCE: Department of Biotechnology, Menarini Ricerche S.p.A., Roma, Italy.
SOURCE: Molecular biotechnology, (1999 Apr) 11 (2) 117-28. Journal code: 9423533. ISSN: 1073-6085.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991013
ED Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991013
AB Two recombinant human granulocyte colony-stimulating factor (rhG-CSF) isoforms were isolated from the medium conditioned by an engineered Chinese hamster ovary (CHO) cell line. The two rhG-CSFs were characterized and were found to differ in the carbohydrate structure attached to Thr-133. The glycoform, referred to as Peak 1, contains the O-linked glycan Neu5Ac(alpha 2-3)Gal(beta 1-3)GalNAc; the Peak 2 glycoform contains the O-linked glycan Neu5Ac(alpha 2-3)Gal(beta 1-3)[Neu5Ac(alpha 2-6)]GalNAc. The two glycoforms displayed a similar biological activity in cultures of a mouse 32D C13 cell line and human bone-marrow myelo-monocytic progenitor cells (CFU-GM). In the latter test both glycoforms displayed a higher activity than nonglycosylated rMet-hG-CSF

from Escherichia coli. The pharmacokinetic profile and activity of the two rhG-CSF glycoforms and of a mixture of them (Pool) were investigated in mice treated with a single injection of rhG-CSF at the doses of 125 micrograms and 250 micrograms/kg, given via the intravenous (i.v.) and the subcutaneous (s.c.) route, respectively. The plasma concentration profiles obtained were similar for all three substances and did not show any relevant differences in absorption or elimination. The pharmacokinetic parameters indicate that the three substances have similar area under the curve (AUCs), volumes of distribution, and terminal half-life. Furthermore, our data indicate a high bioavailability of the two different glycoforms of rhG-CSF when given to mice via the s.c. route either singularly or as a mixture. Detectable levels of rhG-CSF persisted for more than 8 h in the i.v. and more than 24 h in the s.c. route of administration. All three substances induced early neutrophilia in mice. All rhG-CSF-treated mice developed a two-four-fold rise in neutrophil counts as early as 4 h after the intravenous and 2 h after the subcutaneous injection. Relatively high levels of neutrophils were maintained for at least 8 and 24 h after i.v. and s.c. administration, respectively.

L57 ANSWER 3 OF 46 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1998430125 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9757559
TITLE: Purification and characterization of recombinant human granulocyte colony-stimulating factor (rhG-CSF) derivatives: KW-2228 and other derivatives.
AUTHOR: Yamasaki M; Konishi N; Yamaguchi K; Itoh S; Yokoo Y
CORPORATE SOURCE: Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Japan.
SOURCE: Bioscience, biotechnology, and biochemistry, (1998 Aug) 62 (8) 1528-34.
Journal code: 9205717. ISSN: 0916-8451.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981029
ED Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981029
AB Various derivatives of recombinant human granulocyte colony-stimulating factor (rhG-CSF) have been overproduced in Escherichia coli with the strong, inducible trp promoter. A derivative designated as KW-2228 in which the amino acids were replaced at five positions showed more potent granulopoietic activity and stability than those of wild-type both in vitro and in vivo. The purification involved a sequential renaturation process and three-step **chromatography**. Refolding succeeded in very high yield using a urea system. The purity of KW-2228 was greater than 99% as measured by SDS-PAGE and HPLC analysis. According to circular dichroism and nuclear magnetic resonance spectroscopy, rhG-CSF and KW-2228 have very similar conformations. This suggests that the substitution of five amino acids does not appreciably change the conformation of hG-CSF. KW-2228 ([Ala1, Thr3, Tyr4, Arg5, and Ser17]-hG-CSF) and derivative A ([Ala1, Thr3, Tyr4, Arg5]-hG-CSF) are easily crystallized and they show similar in vitro activity. On the other hand, neither rhG-CSF nor derivative B ([Ser17]-hG-CSF) are crystallized under the same conditions.

Thus, the four amino acid substitution (Ala1, Thr3, Tyr4, Arg5) of the N-terminal sequence may facilitate crystallization. The change of Cys17 to Ser may not influence the stability and activity of hG-CSF.

L57 ANSWER 4 OF 46 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 94072865 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7504538
TITLE: Isolation and characterization of three recombinant human granulocyte colony stimulating factor His-->Gln isoforms produced in Escherichia coli.
AUTHOR: Lu H S; Fausset P R; Sotos L S; Clogston C L; Rohde M F; Stoney K S; Herman A C
CORPORATE SOURCE: Amgen, Inc., Amgen Center, Thousand Oaks, California 91320.
SOURCE: Protein expression and purification, (1993 Oct) 4 (5) 465-72.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 19980206
Entered Medline: 19940112

ED Entered STN: 19940203
Last Updated on STN: 19980206
Entered Medline: 19940112

AB This report demonstrates that three variant isoforms of recombinant methionyl human granulocyte colony stimulating factor are present in small quantities in the crude preparation solubilized from Escherichia coli inclusion bodies. These isoforms were separated from the main form of the factor during purification and further isolated by a series of cationic exchange **chromatographic** separations. They exhibit full in vitro biological activity and have slightly lower pI's. Structural characterization of the intact proteins and their isolated peptides by sequence determination and mass spectrometric analysis revealed that they are methionyl granulocyte colony stimulating factors having a His-->Gln replacement at sequence position 53, 157, or 171, respectively. The specific His-->Gln change suggests the occurrence of mistranslation during protein synthesis. These variant forms are **chromatographically** separable during purification and are not detectable in the final purified form of the factor.

L57 ANSWER 5 OF 46 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 91018889 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1699255
TITLE: Purification of a granulocyte colony-stimulating factor from the conditioned medium of a subclone of human bladder carcinoma cell line 5637, HTB9.
AUTHOR: Morioka E; Taniguchi S; Okamura S; Shibuya T; Niho Y
CORPORATE SOURCE: First Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan.
SOURCE: Research in experimental medicine. Zeitschrift fur die gesamte experimentelle Medizin einschliesslich experimenteller Chirurgie, (1990) 190 (4) 229-38.
Journal code: 0324736. ISSN: 0300-9130.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199010
ENTRY DATE: Entered STN: 19910117
Last Updated on STN: 19970203
Entered Medline: 19901031

ED Entered STN: 19910117
Last Updated on STN: 19970203
Entered Medline: 19901031

AB A colony-stimulating factor (CSF) has been purified to homogeneity from the conditioned medium (CM) of a subclone, designated HTB9, of human bladder carcinoma cell line 5637. HTB9 cells were successfully cultured on micro-carrier beads at low-serum concentration, and the resulting CM (HTB9-CM) was concentrated by ultrafiltration. Purification procedures consisted of anion-exchange column **chromatography**, gel-filtration column **chromatography**, and reverse-phase column **chromatography**. The finally purified protein possessed a specific activity more than 10(8) U/mg protein for day-7 CFU-GM colony formation and exhibited a single band representing a molecular weight of 17,000 upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biological activity was apparently specific for a neutrophilic granulocyte lineage of human non-phagocytic bone-marrow cells in vitro, and antiserum raised against this purified protein completely inhibited the activity of recombinant granulocyte colony-stimulating factor.

L57 ANSWER 6 OF 46 MEDLINE on STN
ACCESSION NUMBER: 2000000641 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10531646
TITLE: Improved process for production of recombinant yeast-derived monomeric human G-CSF.
AUTHOR: Bae C S; Yang D S; Lee J; Park Y H
CORPORATE SOURCE: Korea Research Institute of Bioscience and Biotechnology (KRIBB), Yusong, Taejon, South Korea.
SOURCE: Applied microbiology and biotechnology, (1999 Sep) 52 (3) 338-44.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991215

ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991215

AB The human granulocyte colony-stimulating factor (hG-CSF) was efficiently secreted at high levels in fed-batch cultures of recombinant Saccharomyces cerevisiae. However, the secreted recombinant hG-CSF (rhG-CSF) was shown to exist as large multimers in the culture broth due to strong hydrophobic interaction. It was hardly monomerized even by urea at high concentration. This multimer has been reported to diminish specific receptor-binding activity of hG-CSF and causes undesirable problems in the downstream process. When the rhG-CSF was secreted to extracellular broth in the presence of a non-ionic surfactant (Tween 80) in the culture media, the multimerization of the secreted rhG-CSF was efficiently prevented in the fed-batch cultures. Also, the monomer fraction and secreted efficiency of rhG-CSF were significantly increased at the higher culture pH (6.5). Without using any denaturing agents, the secreted rhG-CSF

monomer was easily purified with high recovery yield and purity via a simple purification process under acidic conditions, consisting of diafiltration, cation exchange, and gel filtration **chromatography** . A lyophilization process devoid of intermonomer aggregation was also designed using effective stabilizing agents.

L57 ANSWER 7 OF 46 MEDLINE on STN
ACCESSION NUMBER: 95128084 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7530070
TITLE: Monosaccharide and oligosaccharide analysis of isoelectric focusing-separated and blotted granulocyte colony-stimulating factor glycoforms using high-pH anion-exchange **chromatography** with pulsed amperometric detection.
AUTHOR: Andersen D C; Goochee C F; Cooper G; Weitzhandler M
CORPORATE SOURCE: Department of Chemical Engineering, Stanford University 94305-5025.
SOURCE: Glycobiology, (1994 Aug) 4 (4) 459-67.
Journal code: 9104124. ISSN: 0959-6658.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950307
Last Updated on STN: 19960129
Entered Medline: 19950222

ED Entered STN: 19950307
Last Updated on STN: 19960129
Entered Medline: 19950222

AB In this study, a sensitive, straightforward technique is developed for the analysis of glycoprotein O-linked oligosaccharides. Specifically, O-linked oligosaccharides of granulocyte colony-stimulating factor (G-CSF) are analysed by separating charged glycoforms using isoelectric focusing, electroblotting to polyvinylidene difluoride, releasing monosaccharides and oligosaccharide alditols from the blotted glycoprotein bands, and producing **chromatographs** using high-pH anion-exchange **chromatography** with pulsed amperometric detection. Using this technique, the O-linked structures of G-CSF produced by recombinant Chinese hamster ovary (CHO) cells are deduced by comparison with monosaccharide and oligosaccharide standards. Lectin blotting and peptide sequencing support the identities of the presumed G-CSF glycoforms. The two major glycoforms determined using this methodology correspond to those determined previously for CHO-produced G-CSF using NMR. Additional glycoforms are also identified in this study, presumably resulting from the presence of N-glycolyneuraminic acid in place of N-acetylneuraminic acid. The utility of this analytical approach is then demonstrated in an analysis of the effect of the extracellular environment on the O-linked glycosylation of G-CSF by recombinant CHO cells. Increasing the level of ammonium ion in the culture medium is shown to reduce the percentage of G-CSF produced with sialic acid linked alpha (2,6) to N-acetylgalactosamine.

L57 ANSWER 8 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2004:3053 CAPLUS
DOCUMENT NUMBER: 140:76029
TITLE: Recovery and Purification of recombinant human **granulocyte colony stimulating factor**

INVENTOR(S): Komath, Uma Devi; Lodha, Sanjay; Chigurupati, Jayaram
PATENT ASSIGNEE(S): Reddy's Laboratories Ltd., India; Cord, Janet I.
SOURCE: PCT Int. Appl., 21 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004001056	A1	20031231	WO 2002-US19945	20020624

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: WO 2002-US19945 20020624
ED Entered STN: 02 Jan 2004
AB A simple, economic and scaleable process for the purification of recombinant human **granulocyte colony stimulating factor** (**G-CSF**) expressed in Escherichia coli is provided. The steps include lysing the microorganism, separating the inclusion bodies containing **G-CSF**, a multi step washing procedure for inclusion bodies to remove protein, LPS, and other host cell impurities, refolding the protein at basic pH and cation exchange chromatog.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 9 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2003:491267 CAPLUS
DOCUMENT NUMBER: 139:57953
TITLE: Process for the purification and/or isolation of biologically active **granulocyte colony stimulating factor**
INVENTOR(S): Gaberc Porekar, Vladka; Menart, Viktor
PATENT ASSIGNEE(S): Lek Pharmaceuticals D.D., Slovenia
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003051922	A1	20030626	WO 2002-EP13810	20021205

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

SI 21102	C	20030630	SI 2001-322	20011219
EP 1458757	A1	20040922	EP 2002-804876	20021205
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
BR 2002015191	A	20041116	BR 2002-15191	20021205
PRIORITY APPLN. INFO.:			SI 2001-322 A	20011219
			WO 2002-EP13810 W	20021205

ED Entered STN: 27 Jun 2003

AB The invention relates to the process for the isolation of biol. active **granulocyte colony stimulating factor** (**G-CSF**), which enables the separation of correctly folded biol. active monomeric mols. of **G-CSF** from the incorrectly folded, biol. inactive monomeric, oligo- or polymeric and also from aggregated mols. of **G-CSF** by using immobilized metal affinity chromatog. The process of the invention, if desired the whole process, can be advantageously performed under native conditions. The biol. active **G-CSF** with a purity of greater than 95% is thus obtained. Only 2 addnl. chromatog. steps, cationic exchange chromatog. and gel filtration, are then preferably applied to remove the traces of impurities. The entire process results in the production of higher yields of **G-CSF** with a purity of >99%. The described process is particularly suitable for the industrial production of **G-CSF**.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 10 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2003:487024 CAPLUS

DOCUMENT NUMBER: 139:174792

TITLE: Method of preparing human recombinant **granulocytic colony-stimulating factor**

INVENTOR(S): Romanov, V. P.; Nazarikova, N. I.; Zhdanov, V. V.; Afinogenova, G. N.; Gladchenko, T. N.; Pustoshilova, N. M.; Masycheva, V. I.; Sinichkina, S. A.; Sandakhchiev, L. S.; Gol'dberg, E. D.; Dygai, A. M.; Pozhen'ko, N. S.

PATENT ASSIGNEE(S): Gosudarstvennyi Nauchnyi Tsentr Virusologii i Biotekhnologii "Vektor", Russia

SOURCE: Russ., No pp. given
CODEN: RUXXE7

DOCUMENT TYPE: Patent

LANGUAGE: Russian

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
RU 2201962	C2	20030410	RU 2001-107330	20010319
PRIORITY APPLN. INFO.:			RU 2001-107330	20010319

ED Entered STN: 26 Jun 2003

AB The invention relates to the production of human recombinant **granulocytic colony-stimulating factor** in transformed Escherichia coli cells. Inclusion bodies comprising recombinant factor are dissolved in urea and the reduction reaction is carried

out with 10 mM of 2-mercaptoethanol. Renaturation is carried out by addition of EDTA-containing neutral buffer up to 0.8 M concentration of urea. Chromatog. purification of recombinant factor is carried out on two successively linked columns filled with DEAE-cellulose and SP-Sephadex and the following elution of the end protein from SP-Sephadex column by linear gradient of sodium chloride in 0.02 M sodium acetate buffer, pH 4.4-4.5. The invention allows to increase the yield of product and retain its biol. activity and purity. Invention can be used for isolation and purification of physiol. active human recombinant **granulocytic colony-stimulating factor**.

L57 ANSWER 11 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2004:869412 CAPLUS
DOCUMENT NUMBER: 142:71220
TITLE: Method for purifying active recombinant human **granulocyte-colony stimulating factor**
INVENTOR(S): Jun, Jae Hyeon; Lee, Ho Jin; Lee, Jong Uk; Nam, Jeong Hyeon; Park, Se Cheol; Yoo, Gwang Hyeon
PATENT ASSIGNEE(S): Yuhan Co., Ltd., S. Korea
SOURCE: Repub. Korean Kongkae Taeho Kongbo, No pp. given
CODEN: KRXXA7
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 2001001682	A	20010105	KR 1999-21065	19990608
PRIORITY APPLN. INFO.:			KR 1999-21065	19990608

ED Entered STN: 20 Oct 2004
AB A method for purifying an active recombinant human **granulocyte-colony stimulating factor** (rhG-CSF) is provided which can obtain the active rhG-CSF in a high yield and which meets the biol. unit activity standard (1.0 x 108 Unit/mg) presented in National Institute for Biol. Stds. and Control in purity. Also, the purified rhG-CSF is used as a medicine because it has 0.1 EU/mg/mL or less of an endotoxin, 1.0 ng/mg/mL or less of a host cell-derived peptide, 1.0 pg/mg/mL or less of a host cell-derived DNA, and at least 99 % of the purity in all purification steps. The method for purifying the active rhG-CSF comprises the steps of : carrying out an adsorption chromatog. of the solution, which comprises a solubilization denaturating agent such as SDS and the active rhG-CSF in a form of an inclusion body expressed in a recombinant host cell, to remove the solubilization denaturating agent; mixing an acetic acid with the solution at 1-6 %(volume/volume) of a final concentration followed by carrying out a cation exchange chromatog. using Macro-Prep high S resin and an anion exchange chromatog. using Macro-Prep high Q resin continuously; and carrying out a gel chromatog.

L57 ANSWER 12 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:78076 CAPLUS
DOCUMENT NUMBER: 142:151584
TITLE: Target biological material separation from mixtures using superparamagnetic polysaccharide matrices and formation of the superparamagnetic particles
INVENTOR(S): Marchessault, Robert H.; Shingel, Kirill; Ryan,

Dominic; Llanes, Francisco; Coquoz, Didier G.; Vinson, Robert K.
PATENT ASSIGNEE(S): Can.
SOURCE: U.S. Pat. Appl. Publ., 29 pp., Cont.-in-part of U.S. Ser. No. 352,280.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005019755	A1	20050127	US 2004-765750	20040127
US 2004146855	A1	20040729	US 2003-352280	20030127
PRIORITY APPLN. INFO.:			US 2003-352280 A2	20030127

ED Entered STN: 28 Jan 2005
AB The present invention features a method for preparing superparamagnetic iron particles by the in situ formation of these particles in a cross-linked starch matrix or by the formation of a superparamagnetic chitosan material. The superparamagnetic materials are formed by mild oxidation of ferrous ion, either entrapped into a cross-linked starch matrix or as a chitosan-Fe(II) complex, with the mild oxidizing agent, nitrate, under alkaline conditions. The present invention further features superparamagnetic iron compns. prepared by the method of the invention. The compns. of the invention are useful for the separation, isolation, identification, or purification of biol. materials. Chitosan and FeCl2 were incubated to form a complex, the complex was treated with a solution of NH4OH and then oxidized with KNO3 to prepare superparamagnetic chitosan particles (MagChi). The particles were treated with glutaraldehyde and then reacted with protein A. Sodium cyanoborohydride solution was added to the reaction mixture and incubated overnight. The particles were magnetically separated from unreacted protein in the supernatant. Glycine and sodium cyanoborohydride solution were incubated with the particles for one hour. The resulting MagChi matrix modified by covalent attachment to protein A (MagChi-Protein A) was used to magnetically bind IgG. The MagChi-Protein A matrix showed saturation binding at 2.5 mg of IgG/mg matrix and greater than 90% of the IgG bound could be recovered.

L57 ANSWER 13 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:252530 CAPLUS
DOCUMENT NUMBER: 140:249743
TITLE: Development of purification method of physiol. active proteins (antibodies and cytokines) by using acidic solution from contaminated DNAs and viruses
INVENTOR(S): Takeda, Kozo; Ochi, Norimichi; Ishii, Kimie; Matsuhashi, Manabu; Imamura, Akinori
PATENT ASSIGNEE(S): Chugai Seiyaku Kabushiki Kaisha, Japan
SOURCE: PCT Int. Appl., 32 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004024752	A1	20040325	WO 2003-JP11642	20030911

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: JP 2002-265609 A 20020911
ED Entered STN: 26 Mar 2004
AB A purification method for physiol. active proteins, particularly antibodies (Ig Gs, monoclonal antibody) and cytokines by precipitating contaminants such as DNA and viruses in aqueous solution was developed. The method uses solution (HCl, citric acid or acetic acid solution) of lower pHs than the isoelec. points of the purification target proteins and higher than pH 2.0. The solution used for the process is set-up to be 0 .apprx. 100mM in molarity, 0 .apprx. 0.2 in ion strength and 0 .apprx. 300 mS/m in conductivity In purification of antibodies, protein A or protein G affinity chromatog. matrixes are used with acid elution solution and Tris buffer system to lower the pH. Purifications of anti-human IL-6 receptor antibody, anti-human parathormone related peptide antibody, anti-humanized HM1. 24 antigen antibody and human **G-CSF** by the claimed method have been demonstrated. The final comtamination of DNA and virus can be reduced as low as 22.5 pg DNA/mL and 1.03 log10 virus/mL (the TCID50 method) after purification
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 14 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:97452 CAPLUS
DOCUMENT NUMBER: 138:149953
TITLE: Preparation and purification of recombinant proteins from fusion proteins containing ubiquitin cleavage site by using the change of isoelectric point
INVENTOR(S): Lee, Woo-Jong; Park, Heung-Bok; Cho, Tae-Hoon; Kim, Jeong-Min; Park, Yeon-Sung
PATENT ASSIGNEE(S): Samyang Genex Corporation, S. Korea
SOURCE: PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003010204	A1	20030206	WO 2002-KR1416	20020726

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,

NE, SN, TD, TG

KR 2003010536	A	20030205	KR 2002-43968	20020725
EP 1417237	A1	20040512	EP 2002-753269	20020726
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
JP 2005506319	T2	20050303	JP 2003-515562	20020726
PRIORITY APPLN. INFO.:			KR 2001-45229 A	20010726
			KR 2002-43968 A	20020725
			WO 2002-KR1416 W	20020726

ED Entered STN: 07 Feb 2003

AB The present invention relates to a process of separation a protein of interest comprising expressing the protein of interest fused with a fusion partner, selectively adsorbing on matrix, and performing efficient cleavage reaction on the fusion protein adsorbed on matrix or separated from the matrix, and recovering the protein of interest. Also, the present invention provides a fusion protein comprising a protein of interest and a fusion partner, wherein the fusion partner comprises an amino acid sequence which can be cleaved by ubiquitin cleavage enzyme at its C-terminus, and which has a difference in isoelec. point of 1 or more from the protein of interest. The ubiquitin cleavage site is ubiquitin or peptide derived from ubiquitin. The fusion partner is glutathione S-transferase, maltose-binding protein or thioredoxin. According to the present invention, the proteins of interest can be purified in high yield and purity. In addition, complicated separating processes required in the production of recombinant proteins can be eliminated so as to cut down the production cost. Exemplary production and purification of human growth hormone and interferon using fusion proteins with ubiquitin is described.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 15 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:696617 CAPLUS

DOCUMENT NUMBER: 139:225485

TITLE: Methods for cloning, synthesis and purification of human growth hormone and **granulocyte colony stimulating factor** in plants

INVENTOR(S): Russell, Douglas A.; Schlittler, Michael

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 53 pp., Cont.-in-part of U.S. Ser. No. 316,847, abandoned.
CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003167531	A1	20030904	US 2001-824200	20010403
US 2002053094	A1	20020502	US 1998-113244	19980710
US 6512162	B2	20030128		
US 2003033636	A1	20030213	US 2002-103516	20020320
US 6812379	B2	20041102		
PRIORITY APPLN. INFO.:			US 1998-113244 A2	19980710
			US 1999-316847 B2	19990521
			US 2000-194217P P	20000403

ED Entered STN: 05 Sep 2003
AB The invention provides a detailed process to express human growth hormone (hGH) and **granulocyte colony stimulating factor** (**G-CSF**) in plants. Several signal peptides were fused with hGH and **G-CSF** for targeting and accumulation of expressed polypeptides into desired subcellular location such as cytosol, plasmid and endoplasmic reticulum. The invention also provides detailed description of purification of hGH and **G-CSF** from transgenic corn and tobacco plant and cell lines. The mol. weight of authentic hGH and **G-CSF** are 21,255 and 18814 resp. determined by mass spectrometry. The hGH and **G-CSF** purified from plant show close bioactivity with standard hGH and **G-CSF** in stimulation the growth of rat. The invention also provides the process of large scale purification of hGH and **G-CSF** which can be used in pharmaceutical industry to produce cytokine for clin. purpose.

L57 ANSWER 16 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:538300 CAPLUS
DOCUMENT NUMBER: 139:208799
TITLE: Method for preparing human **granulocyte colony-stimulating factor** (hG-CSF) secreted into the milk of transgenic animals
INVENTOR(S): Prokof'ev, M. I.; Gorodetskii, S. I.; Kosorukov, V. S.; Mezina, M. N.; Bukreev, Yu. M.; Baryshnikov, A. Yu.
PATENT ASSIGNEE(S): OOO "Novenergo", Russia; Kosorukov, Vyacheslav Stanislavovich
SOURCE: Russ., No pp. given
CODEN: RUXXE7
DOCUMENT TYPE: Patent
LANGUAGE: Russian
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
RU 2207373	C1	20030627	RU 2002-111017	20020425
PRIORITY APPLN. INFO.:			RU 2002-111017	20020425

ED Entered STN: 15 Jul 2003
AB The invention relates to a method for preparing human **granulocytic colony-stimulating factor** (hG-CSF) in the milk of transgenic animals. Rabbits with human hybrid gene h-GM-1 that comprises a genomic copy of the gene encoding human **granulocytic colony-stimulating factor** and regulatory elements of milk genes directing the secretion of hG-CSF protein into milk. The method allows to prepare the purified protein hG-CSF in a homogeneous state that is useful for further use in pharmacol. and for preparing medicinal formulation of the preparation hG-CSF.

L57 ANSWER 17 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:766974 CAPLUS
DOCUMENT NUMBER: 140:180174
TITLE: High level expression, purification, and in vivo activity of bovine **granulocyte-colony stimulating factor** produced using a baculovirus system
AUTHOR(S): Nagata, Tomoshi; Tohda, Yukihiko; Yokomizo, Yuichi; Nakamura, Masayuki; Takehara, Kazuaki

CORPORATE SOURCE: School of Veterinary Medicine and Animal Sciences, Laboratory of Poultry Diseases, Kitasato University, Towada, Aomori, 034-8628, Japan
SOURCE: Veterinary Immunology and Immunopathology (2003), 96(1-2), 105-110
CODEN: VIIMDS; ISSN: 0165-2427
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 01 Oct 2003
AB A bovine **granulocyte-colony stimulating factor** (bG-CSF) cDNA clone bearing a C-terminal poly-His-tag (bG-CSFHis) was constructed and expressed by the baculovirus expression system. The bG-CSFHis was expressed as an approx. 19 kDa protein in the culture supernatants and was purified using a nickel chelate column. The purified bG-CSFHis had bioactivity in vitro in the NFS-60 bioassay. In order to evaluate activity in vivo, purified bG-CSFHis was administered to cattle as single or multiple dosages. The bG-CSFHis increased neutrophil counts in peripheral blood and modulated the phagocytic activity of the neutrophils. The data indicates that the recombinant protein had activity in vivo.
REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 18 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:294412 CAPLUS
DOCUMENT NUMBER: 139:1089
TITLE: Mutation of surface-exposed histidine residues of recombinant human **granulocyte-colony stimulating factor** (Cys17Ser) impacts on interaction with chelated metal ions and refolding in aqueous two-phase systems
AUTHOR(S): Zaveckas, Mindaugas; Luksa, Virginijus; Zvirblis, Gintautas; Chmieliauskaite, Valerija; Bumelis, Vladas; Pesliakas, Henrikas
CORPORATE SOURCE: Institute of Biotechnology, Vilnius, LT-2028, Lithuania
SOURCE: Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences (2003), 786(1-2), 17-32
CODEN: JCBAAI; ISSN: 1570-0232
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 16 Apr 2003
AB Site directed mutagenesis of Cys 17→Ser 17 form of recombinant human **granulocyte colony stimulating factor** (rhG-CSF C17S) for sequential replacing of surface His43 and His52 with alanine was used to identify residues critical for the protein interaction with metal ions, in particular Ni2+ chelated by dye Light Resistant Yellow 2 KT (LR Yellow 2KT)-polyethyleneglycol (PEG), and refolding after partitioning of inclusion bodies in aqueous two-phase systems. Strong binding of rhG-CSF (C17S) to PEG-LR Yellow 2KT-Cu(II) complex allowed for the adoption of affinity chromatog. on Sepharose-LR Yellow 2KT-Cu(II) that appeared to be essential for the rapid isolation of mutated forms of rhG-CSF. Efficiency of that purification stage is exemplified by isolation of rhG-CSF (C17S, H43A) and rhG-CSF (C17S, H43A, H52A) mutants in correctly folded and highly purified state. Affinity partitioning of rhG-CSF histidine mutants was studied in aqueous two-phase

systems containing Cu(II), Ni(II) and Hg(II) chelated by LR Yellow 2KT-PEG at pH 7.0 and Cu(II)-at pH 5.0. It was determined, that affinity of rhG-CSF mutants for metal ions decreased in the order of C17S > C17S, H43A > C17S, H43A, H52A for Cu(II), and C17S = C17S, H43A > C17S, H43A, H52A for Ni(II) ions, while affinity of all rhG-CSF mutants for Hg(II) ions was of the same order of magnitude. Influence of His43 and His52 mutation on protein refolding was studied by partitioning of the resp. inclusion body extract in aqueous two-phase systems containing Ni(II) and Hg(II) ions. Data on rhG-CSF histidine mutant partitioning and refolding indicated, that His52 mutation is crucial for the strength of protein interaction with chelated Ni(II) ions and refolding efficiency.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 19 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:716305 CAPLUS
DOCUMENT NUMBER: 137:228936
TITLE: Method for purifying protein
INVENTOR(S): Takeda, Kozo; Ochi, Norimichi
PATENT ASSIGNEE(S): Chugai Seiyaku Kabushiki Kaisha, Japan
SOURCE: PCT Int. Appl., 24 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002072615	A1	20020919	WO 2002-JP2248	20020311
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1380589	A1	20040114	EP 2002-703958	20020311
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2004138424	A1	20040715	US 2003-471374	20030909
PRIORITY APPLN. INFO.:			JP 2001-67111 A	20010309
			WO 2002-JP2248 W	20020311

ED Entered STN: 20 Sep 2002
AB A method is provided for removing DNA impurity in a sample containing a physiol. active protein, and thereby, purifying the protein. The method comprises a step for treating the physiol. active protein-containing sample so as to give an aqueous solution with low conductivity at the pH in a neutral region, and
a step for removing the particles generated.
REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 20 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:704270 CAPLUS
DOCUMENT NUMBER: 138:319732

TITLE: Two-step chromatography - a unique procedure for purification of **granulocyte colony stimulating factor** (**G-CSF**) from recombinant E.coli
AUTHOR(S): Chakraborty, Chiranjib; Bhattacharyya, Atanu
CORPORATE SOURCE: Department of Medical Science and Biotechnology, Macleods Pharmaceuticals Ltd., Mumbai, 400059, India
SOURCE: Journal of Ecophysiology & Occupational Health (2002), 2(1 & 2), 135-142
CODEN: JEOHAG; ISSN: 0972-4397
PUBLISHER: Academy of Environmental Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 17 Sep 2002
AB Recombinant **granulocyte colony stimulating factor** (**G-CSF**) is used nowadays for efficacy in preventing infectious complications of some neutropenic states, accelerate neutrophil recovery from myelosuppressive treatments. **G-CSF** decreases the morbidity of cancer chemotherapy by reducing the incidence of febrile neutropenia, the morbidity of high-dose chemotherapy supported by bone marrow transplantation. This paper describes the method of 2-step chromatog. procedure (gel filtration and ion-exchange) for purification of **G-CSF** from recombinant E. coli.
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 21 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:731048 CAPLUS
DOCUMENT NUMBER: 135:283966
TITLE: Plasmid vectors and recombinant production of human **granulocyte colony stimulating factor** (**G-CSF**) in Escherichia coli
INVENTOR(S): Lee, Sang-Yup; Jeong, Ki-Jun
PATENT ASSIGNEE(S): Korea Advanced Institute of Science and Technology, S. Korea
SOURCE: PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001073081	A1	20011004	WO 2001-KR549	20010331
W: CN, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
KR 2001094652	A	20011101	KR 2000-17052	20000331
EP 1185675	A1	20020313	EP 2001-922090	20010331
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 2003153049	A1	20030814	US 2001-9792	20011213
PRIORITY APPLN. INFO.:			KR 2000-17052 A	20000331
			WO 2001-KR549 W	20010331

ED Entered STN: 07 Oct 2001
AB The invention relates to construction of plasmid vectors and methods to

recombinant production of human **granulocyte colony stimulating factor** (**G-CSF**) in E.coli. The present invention provides a recombinant plasmid vector comprising a kanamycin resistance gene, a promoter, an endoxylanase signal sequence, a nucleotide sequence coding for an oligopeptide consisting of 13 amino acids including 6 consecutive histidine residues, and a human **granulocyte colony stimulating factor** (hG-CSF) gene. The lower G-C content in N-terminal portion of hG-CSF is created due to inhibitory effect of high G+C content in N-terminal of hG-CSF on transcription and translation. The signal sequence od endoxylanase derived from Bacillus sp. is used for the secretion of hG-CSF protein from E.coli. The present invention also provides an E. coli transformed with the said vector and a process for producing complete hG-CSF protein with high purity from the protein pool secreted by the said microorganism. In accordance with the invention, the hG-CSF protein can be prepared with high purity through rather simple process facilitating secretion of large amount of hG-CSF fusion protein into the periplasm, which does not require complicated processes such as solubilization and subsequent refolding required for isolation of the hG-CSF protein produced in cytoplasm as insol. inclusion bodies by conventional techniques. The hG-CSF protein prepared by this methods can be widely used as an active ingredient in the development of supplementary agents for anticancer therapy.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 22 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:50679 CAPLUS
DOCUMENT NUMBER: 134:99561
TITLE: Chromatographic method for purifying **granulocyte-colony stimulating factor** (**G-CSF**)
INVENTOR(S): Dumas, Jacques; Rey, Lucien; Sarubbi, Edoardo
PATENT ASSIGNEE(S): Hoechst Marion Roussel, Fr.
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001004154	A1	20010118	WO 2000-FR1937	20000706
W: AE, AG, AL, AU, BA, BB, BG, BR, BZ, CA, CN, CR, CU, CZ, DM, DZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
FR 2796071	A1	20010112	FR 1999-8831	19990708
FR 2796071	B1	20010907		
CA 2378566	AA	20010118	CA 2000-2378566	20000706
EP 1200471	A1	20020502	EP 2000-949647	20000706
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
JP 2003504378	T2	20030204	JP 2001-509763	20000706

PRIORITY APPLN. INFO.: FR 1999-8831 A 19990708
WO 2000-FR1937 W 20000706

ED Entered STN: 19 Jan 2001

AB The invention provides a method for purifying **G-CSF** from a biol. sample comprising: (a) reducing the volume of the biol. sample containing the **G-CSF** by hydrophobic interaction chromatog. to obtain a concentrated, desalted and enriched fraction; (b) passing the concentrated fraction over hydroxyapatite in conditions whereby the **G-CSF** is slightly bound to obtain a concentrated, desalted and enriched fraction containing the **G-CSF**; and (c) collecting the **G-CSF**.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 23 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:50519 CAPLUS
DOCUMENT NUMBER: 134:130268
TITLE: Immunoglobulin fusion proteins
INVENTOR(S): Cox, George N., III; Doherty, Daniel H.
PATENT ASSIGNEE(S): Bolder Biotechnology Inc., USA
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001003737	A1	20010118	WO 2000-US19336	20000713
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2379388	AA	20010118	CA 2000-2379388	20000713
EP 1200124	A1	20020502	EP 2000-947408	20000713
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
JP 2003508023	T2	20030304	JP 2001-509211	20000713
NZ 517184	A	20040227	NZ 2000-517184	20000713

PRIORITY APPLN. INFO.: US 1999-143458P P 19990713
WO 2000-US19336 W 20000713

ED Entered STN: 19 Jan 2001

AB The present invention relates to novel methods for making fusion proteins comprising a cytokine or growth factor fused to an Ig domain. The growth factor/cytokine can be fused directly to an Ig domain or through a peptide linker. The purified growth factor/cytokine-IgG fusion proteins produced by the novel methods are biol. active and can be used to treat diseases for which the non-fused growth factor/cytokine are useful.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 24 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:931760 CAPLUS
DOCUMENT NUMBER: 137:135688
TITLE: Excretion of hG-CSF into culture medium of Escherichia coli
AUTHOR(S): Wang, Fuqiang; Zhou, Xingjun; Wang, Yanfang; Tian, Xuehong; Zhang, Qian; He, Bingkun
CORPORATE SOURCE: New Drug R + D Center, North China Pharmaceutical Group Corporation, Shijiazhuang, 050015, Peop. Rep. China
SOURCE: Yaowu Shengwu Jishu (2001), 8(5), 260-263
CODEN: YSJIFO; ISSN: 1005-8915
PUBLISHER: Yaowu Shengwu Jishu Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
ED Entered STN: 27 Dec 2001
AB Escherichia coli was generally not considered to be an appropriate host for the excretion of proteins into the culture. A hG-CSF (human **granulocyte colony-stimulating factor**) expression vector pSEGF was constructed and the human **G-CSF** excreted successfully into the E. coli culture medium. In the system, hG-CSF gene fused to two IgG-binding domains of Staphylococcus aureus protein A and controlled by both lac promoter and S. aureus protein A promoter for expression. The Staphylococcal protein A signal peptide was used for secretion of the foreign protein. The expressed hGG-CSF was soluble and easily excreted to the culture medium of E. coli. Bioassay showed the culture medium revealed the hG-CSF biol. activity. The fusion protein might be purified directly from the culture medium by affinity chromatog. on IgG sepharose. A protease Factor Xa recognition site (-IEGR-) was designed between the ZZ domains and the N-terminal domain of hG-CSF. The factor Xa was used to cleave the fusion product to generate mature hG-CSF with the same primary structure as natural hG-CSF.

L57 ANSWER 25 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:73048 CAPLUS
DOCUMENT NUMBER: 136:84763
TITLE: Process for reproducing human **granule-colony stimulating factor** (hG-CSF) in type of inclusion body by gel filtration column chromatography
INVENTOR(S): Lee, Jong Wuk; Yu, Gwang Hyeon; Jun, Jae Hyeon; Park, Se Cheol; Nam, Jeong Hyeon; Lee, Ho Jin
PATENT ASSIGNEE(S): Yuhan Co., Ltd., S. Korea
SOURCE: Repub. Korean Kongkae Taeho Kongbo, No pp. given
CODEN: KRXXA7
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 2000021608	A	20000425	KR 1998-40784	19980930

PRIORITY APPLN. INFO.: KR 1998-40784 19980930
ED Entered STN: 28 Jan 2002
AB A process for reproducing a human **granule-colony stimulating factor** (hG-CSF) is provided which can reduce a reaction volume and obtain a high yield of the hG-CSF by using an inclusion body of the hG-CSF, so it is easy to produce the hG-CSF in a great quantity. The process comprises suspending the inclusion body of

the hG-CSF expressed from a recombinant strain which is cultivated in a solution containing DL-Dithiothritol (DTT) and SDS; adjusting pH of the culture solution to an alkali condition; passing the culture solution through a gel filtration column chromatog. equilibrated with a buffer solution containing SDS and Cu2+; and recovering a fraction of the reproducing hG-CSF.

L57 ANSWER 26 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:231763 CAPLUS
DOCUMENT NUMBER: 134:227359
TITLE: Capsules containing Achyranthes bidentata polysaccharide and process for extracting granulocyte stimulating factor
INVENTOR(S): Ye, Yuchun; Qian, Guangyu
PATENT ASSIGNEE(S): Jianshen Bio-Engineering Co., Ltd., Shanghai, Peop. Rep. China
SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, 15 pp.
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1251772	A	20000503	CN 1999-116917	19990927
CN 1101702	B	20030219		
PRIORITY APPLN. INFO.:			CN 1999-116917	19990927

ED Entered STN: 03 Apr 2001
AB The capsule is composed of Achyranthes bidentata polysaccharide 125, lyophilized goat embryo powder 119, goat embryo hydrolyzate 3, and cultured goat embryonic lyophilized powder 1 mg. The goat embryo hydrolyzate is prepared by digesting goat embryonic tissue with enzyme, precipitating with 75% ethanol, dewatering, and drying in vacuum. Granulocyte-stimulating factor is prepared by collecting fresh goat embryo, slicing, culturing in fetal bovine serum- containing RPMI-1640 culture medium for 7-10 days, centrifugating, ultrafiltering supernatant to obtain crude product; concentrating with XHP-1 ultrafilter membrane, dialyzing in 0.02M acetate buffer for 48 h, regulating with acetic acid to pH 4.0, centrifugating, and purifying supernatant successively on CM-52 column and Sephadex G-75 column.

L57 ANSWER 27 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:876453 CAPLUS
DOCUMENT NUMBER: 134:95604
TITLE: Comparative studies of recombinant human **granulocyte-colony stimulating factor**, its Ser-17 and (His)6-tagged forms interaction with metal ions by means of immobilized metal ion affinity partitioning. Effect of chelated nickel and mercuric ions on extraction and refolding of proteins from inclusion bodies
AUTHOR(S): Zaveckas, M.; Baskeviciute, B.; Luksa, V.; Zvirblis, G.; Chmieliauskaite, V.; Bumelis, V.; Pesliakas, H.
CORPORATE SOURCE: Institute of Biotechnology, Vilnius, 2028, Lithuania
SOURCE: Journal of Chromatography, A (2000), 904(2), 145-169
CODEN: JCRAEY; ISSN: 0021-9673
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal

LANGUAGE: English
ED Entered STN: 15 Dec 2000
AB The chelation capability of the reactive dye Light Resistant Yellow 2KT towards metal ions, particularly mercury(II) was evaluated in the pH range 5.0-7.0, and it was shown that the dye-Hg(II) complex has a free site for the interaction with human recombinant **granulocyte-colony stimulating factor** (rhG-CSF) from Escherichia coli. Affinity partitioning of three rhG-CSF forms - native, rhG-CSF[Ser17] and (His)6-rhG-CSF was studied in aqueous two-phase systems, which contained metal ions - Cu(II), Ni(II) and Hg(II) - chelated by dye-poly(ethylene glycol) at pH 5.0 and 7.0, in the presence or absence of many selected agents. It was determined, that chelated Ni(II) ions exhibited stronger interaction with the hexahistidine-tagged protein form, while the extraction power of Cu(II) ions was found to be of comparable order of magnitude for all three protein forms at pH 7.0. A comparative study of rhG-CSF and both its forms partitioning in the presence of chelated Hg(II) ions at pH 7.0 and 5.0 revealed possible direct interaction between Hg(II) ions and unpaired Cys-17 of rhG-CSF. The partitioning of three rhG-CSF forms inclusion body extract was studied in the presence of chelated Ni(II) and Hg(II) ions thus explaining the efficiency of targeted proteins renaturation gained upon their inclusion body forms interactions with chelated metal ions.
REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 28 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:595213 CAPLUS
DOCUMENT NUMBER: 131:213188
TITLE: A process for isolating and purifying viruses, soluble proteins and peptides from plant sources including transgenic plants
INVENTOR(S): Garger, Stephen J.; Holtz, R. Barry; Mcculloch, Michael J.; Turpen, Thomas H.
PATENT ASSIGNEE(S): Biosource Technologies, Inc., USA
SOURCE: PCT Int. Appl., 58 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9946288	A2	19990916	WO 1999-US5056	19990309
WO 9946288	A3	20000120		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6037456	A	20000314	US 1998-37751	19980310
US 6033895	A	20000307	US 1999-259741	19990225
CA 2322616	AA	19990916	CA 1999-2322616	19990309
AU 9930725	A1	19990927	AU 1999-30725	19990309
AU 747647	B2	20020516		
EP 1062235	A2	20001227	EP 1999-912327	19990309

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

JP 2002506080	T2	20020226	JP 2000-535664	19990309
US 6303779	B1	20011016	US 1999-466422	19991217
US 2003049813	A1	20030313	US 2001-962527	20010924
US 6740740	B2	20040525		
US 2004166026	A1	20040826	US 2004-781448	20040218
US 2004171813	A1	20040902	US 2004-828029	20040420

PRIORITY APPLN. INFO.:

US 1998-37751	A	19980310
US 1999-259741	A1	19990225
WO 1999-US5056	W	19990309
US 1999-466422	A1	19991217
US 2001-962527	A1	20010924
US 2001-970150	A3	20011003

ED Entered STN: 21 Sep 1999

AB The present invention features a method for isolating and purifying viruses, proteins and peptides of interest from a plant host which is applicable on a large scale. Moreover, the present invention provides a more efficient method for isolating viruses, proteins and peptides of interest than those methods described in the prior art. In general, the present method of isolating viruses, proteins and peptides of interest comprises the steps of homogenizing a plant to produce a green juice, adjusting the pH of and heating the green juice, separating the target species, either virus or protein/peptide, from other components of the green juice by one or more cycles of centrifugation, resuspension, and ultrafiltration, and finally purifying virus particles by such procedure as PEG-precipitation or purifying proteins and peptides by such procedures as chromatog. and/or salt precipitation The invention also concerns transgenic plants and the isolation of viral proteins and/or other fusion proteins.

L57 ANSWER 29 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:855250 CAPLUS
DOCUMENT NUMBER: 142:100271
TITLE: Method of purification of active human **granulocyte colony stimulating factor**
INVENTOR(S): Kang, Soo Hyung; Park, Choong Il; Park, Jang Hyun; Na, Kyu Hum
PATENT ASSIGNEE(S): Dong-A Pharm. Co., Ltd., S. Korea
SOURCE: Repub. Korea, No pp. given
CODEN: KRXXFC
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 212071	B1	19990802	KR 1994-25767	19941008

PRIORITY APPLN. INFO.: KR 1994-25767 19941008

ED Entered STN: 18 Oct 2004

AB A process for purifying an activated human **granulocyte colony stimulating factor**(hG-CSF) is provided for increasing purity of activated hG-CSF prior to purification process and simplifying the purification steps, so that the production cost can be lowered. The process for purifying an activated hG-CSF comprises the steps of: activating a solution of human **granulocyte colony stimulating factor** expressed from the host cells; adjusting pH of the activated hG-CSF solution to 4.0-7.0 to precipitate impurities

and centrifuging the solution; collecting the supernatant and performing ion-exchange chromatog.; adding 30% of ammonium hydrosulfate into the solution to precipitate impurities; suspending ppts. into a 10 mM sodium acetate solution containing 0.04% of polysorbate and dialyzing the suspended solution; performing gel-filtration chromatog. with the dialyzed solution to purify the activated hG-CSF.

L57 ANSWER 30 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:446492 CAPLUS
DOCUMENT NUMBER: 131:225618
TITLE: The separation efficiency of biopolymers with short column in liquid chromatography
AUTHOR(S): Liu, Tong; Geng, Xin Du
CORPORATE SOURCE: Institute of Modern Separation Science, Shaanxi Provincial Key Laboratory of Modern Separation Science, Northwest University, Xi'an, 710069, Peop. Rep. China
SOURCE: Chinese Chemical Letters (1999), 10(3), 219-222
CODEN: CCLEE7; ISSN: 1001-8417
PUBLISHER: Chinese Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 21 Jul 1999
AB The separation efficiency of biopolymers with a short column in liquid chromatog. has been investigated in this paper. It was found that the column length has slight effect on the resolution of biopolymers under gradient elution. The reasons have been explained by stoichiometric displacement model for retention of solute. The column 1.0 cm long was also used in the separation and purification of recombinant human granulocyte colony-simulating factor (rhG-CSF). It only took 40 min and the purity by one step was found to be almost 100%.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L57 ANSWER 31 OF 46 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:31707 CAPLUS
DOCUMENT NUMBER: 132:59604
TITLE: Preparation of recombinant human **granulocyte colony stimulation factor**
INVENTOR(S): Su, Yong; Kong, Tieshan; Wang, Changmei; Huang, Yanshan; Sun, Handong
PATENT ASSIGNEE(S): Jiuyuan Gene Engineering Co., Ltd., Hangzhou, Peop. Rep. China
SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, 15 pp.
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1167150	A	19971210	CN 1996-106418	19960605
CN 1083488	B	20020424		
PRIORITY APPLN. INFO.:			CN 1996-106418	19960605

ED Entered STN: 14 Jan 2000
AB The process comprises preparation of the cDNA for **granulocyte**

colony stimulation factor (G-**CSF**) from human peripheral leukocyte by RT-PCR, construction of recombinant plasmid, expression of the cDNA in Escherichia coli, renaturation by dialyzing with external-or inner-pressure type hollow fiber, and repetitively purification with ion-exchange column, hydrophobic column, and mol. sieve column. The hollow fiber is selected from cellulose acetate, cellulose nitrate, and polysulfone; the filler for ion-exchange column from DEAE, Q, and QAE; that for hydrophobic column from the hydrophobic medium containing butyl-, phenyl-, or octyl- group; and that for the mol. sieve column from Sephacryl series, and Superdex series. The mobile phase for ion-exchange column is composed of mobile phase I, and II. Mobile phase I is selected from 20-50 mM imidazole HCl, barbitone HCl, Tris HCl, and borax-boric acid or H3PO4 buffer solution of pH 7.0-9.0; mobile phase II as gradient eluent is composed of the mobile phase I, and 0.01-0.5N NaCl solution The mobile phase for hydrophobic column is selected to resp. use the mobile phase I containing 0.1-1.0 N (NH4)2SO4, or 0.1-1.0 N NaCl, mobile phase I, and water. The mobile phase for mol. sieve column is 5-50 mM acetic acid-Na acetate.

L57 ANSWER 32 OF 46 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 6

ACCESSION NUMBER: 2001281530 EMBASE
TITLE: **Isolation**, nucleotide sequence and expression of a cDNA encoding feline granulocyte colony-stimulating factor.
AUTHOR: Dunham S.P.; Onions D.E.
CORPORATE SOURCE: S.P. Dunham, Retrovirus Research Laboratory, Department of Veterinary Pathology, University of Glasgow, Bearsden Road, Glasgow G61 1QH, United Kingdom. s.dunham@vet.gla.ac.uk
SOURCE: Cytokine, (21 Jun 2001) Vol. 14, No. 6, pp. 347-351.
Refs: 15
ISSN: 1043-4666 CODEN: CYTIE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20010830
Last Updated on STN: 20010830
ED Entered STN: 20010830
Last Updated on STN: 20010830

AB A cDNA encoding feline granulocyte colony stimulating factor (fG-CSF) was cloned from alveolar macrophages using the reverse transcriptase-polymerase chain reaction. The cDNA is 949 bp in length and encodes a predicted mature protein of 174 amino acids. Recombinant fG-CSF was expressed as a glutathione S-transferase fusion and purified by affinity **chromatography**. Biological activity of the recombinant protein was demonstrated using the murine myeloblastic cell line GNFS-60, which showed an ED(50) for fG-CSF of approximately 2 ng/ml. .COPYRGT. 2001 Academic Press.

L57 ANSWER 33 OF 46 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 92245933 EMBASE
DOCUMENT NUMBER: 1992245933
TITLE: Pharmacokinetic and pharmacodynamic comparisons between human granulocyte colony-stimulating factor **purified** from human bladder carcinoma cell line

5637 culture medium and recombinant human granulocyte colony-stimulating factor produced in Escherichia coli.
AUTHOR: Tanaka H.; Kaneko T.
CORPORATE SOURCE: Pharmaceutical Development Lab., Kirin Brewery Co., Ltd., 2-2, Soujamachi 1-chome,Maebashi-shi, Gunma 371, Japan
SOURCE: Journal of Pharmacology and Experimental Therapeutics, (1992) Vol. 262, No. 1, pp. 439-444.
ISSN: 0022-3565 CODEN: JPETAB
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 920912
Last Updated on STN: 920912
ED Entered STN: 920912
Last Updated on STN: 920912
AB The pharmacokinetics and biological activities of recombinant human granulocyte colony-stimulating factor (hG-CSF) produced in Escherichia coli were compared with those of hG-CSF purified from human bladder carcinoma cell line 5637 culture medium (5637-hG-CSF). Recombinant hG-CSF was biologically active in a bone marrow cell proliferation assay in vitro, with a dose- response curve similar to that of 5637-hG-CSF. The effects of 5637- and recombinant hG-CSF administered via i.v. injection to rats showed similar response patterns of neutrophil counts in peripheral blood. From these results, it is concluded that the O-linked sugar chain of hG-CSF does not contribute to the in vitro and in vivo biological activities. The pharmacokinetics of both forms of hG-CSF in rats were investigated using a sandwich enzyme-linked immunosorbent assay. After i.v. administration, the serum concentration-time curves of 5637- and recombinant hG-CSF declined biexponentially. Total body clearance and steady-state volume of distribution of 5637-hG-CSF were smaller than those for the recombinant form. After s.c. administration, a lower peak serum level, smaller AUC, and lower bioavailability of 5637-hG-CSF were observed compared to recombinant hG-CSF.

L57 ANSWER 34 OF 46 EMBASE
on STN
ACCESSION NUMBER: 90346003 EMBASE
DOCUMENT NUMBER: 1990346003
TITLE: Expression and **purification** of lymphokines.
AUTHOR: Sassenfeld H.
CORPORATE SOURCE: Process Development, Immunex Corp., Seattle, WA, United States
SOURCE: Drug News and Perspectives, (1990) Vol. 3, No. 2, pp. 90-94.
ISSN: 0214-0934 CODEN: DNPEED
COUNTRY: Spain
DOCUMENT TYPE: Journal; (Short Survey)
FILE SEGMENT: 025 Hematology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
ENTRY DATE: Entered STN: 911213

Last Updated on STN: 911213
ED Entered STN: 911213
Last Updated on STN: 911213

L57 ANSWER 35 OF 46 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 89020041 EMBASE
DOCUMENT NUMBER: 1989020041
TITLE: Hydrophobic **retention** of proteins using high-performance size-exclusion **chromatography**.
AUTHOR: Watson E.; Kenney W.C.
CORPORATE SOURCE: Amgen Inc., Thousand Oaks, CA 91320, United States
SOURCE: Biotechnology and Applied Biochemistry, (1988) Vol. 10, No. 6, pp. 551-554.
ISSN: 0885-4513 CODEN: BABIEC
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
ENTRY DATE: Entered STN: 911212
Last Updated on STN: 911212
ED Entered STN: 911212
Last Updated on STN: 911212

L57 ANSWER 36 OF 46 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 86196086 EMBASE
DOCUMENT NUMBER: 1986196086
TITLE: **Purification** to homogeneity of a human hematopoietic growth factor that stimulates the growth of a murine interleukin 3-dependent cell line.
AUTHOR: Watson J.D.; Crosier P.S.; March C.J.; et al.
CORPORATE SOURCE: Department of Immunobiology, School of Medicine, University of Auckland, Auckland, New Zealand
SOURCE: Journal of Immunology, (1986) Vol. 137, No. 3, pp. 854-857.
CODEN: JOIMA3
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 026 Immunology, Serology and Transplantation
025 Hematology
030 Pharmacology
LANGUAGE: English
ENTRY DATE: Entered STN: 911210
Last Updated on STN: 911210
ED Entered STN: 911210
Last Updated on STN: 911210
AB A human lymphokine derived from the 5637 bladder carcinoma has been purified to homogeneity by using sequential reverse-phase high pressure liquid **chromatography**. A high recovery of biological activity is obtained by using this purification. The NH2-terminal amino acid sequence shows no homology to human interleukin 1 (IL 1), human IL 2, murine IL 3, or human granulocyte-macrophage colony-stimulating factor. The growth-promoting properties of the 5637-derived factor can be rapidly assayed by using the murine IL 3-dependent 32D c1-23 cell line. The amino acid sequence described is identical to that recently described for a human granulocyte colony-stimulating factor.

L57 ANSWER 37 OF 46 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 85078255 EMBASE
DOCUMENT NUMBER: 1985078255
TITLE: **Purification** and partial amino acid sequence of asialo murine granulocyte-macrophage colony stimulating factor.
AUTHOR: Sparrow L.G.; Metcalf D.; Hunkapiller M.W.; et al.
CORPORATE SOURCE: Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organisation, Parkville 3052, Vic., Australia
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1985) Vol. 82, No. 2, pp. 292-296.
CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
ENTRY DATE: Entered STN: 911210
Last Updated on STN: 911210
ED Entered STN: 911210
Last Updated on STN: 911210
AB A procedure utilizing reversed-phase high-performance liquid **chromatography** is described for the purification of asialo granulocyte-macrophage colony stimulating factor (asialo-GM-CSF) from mouse lung-conditioned medium. In the purification, the partially purified factor was treated with neuraminidase to reduce charge heterogeneity due to variable degrees of sialation. Three active forms of the asialo factor were separated by the final reversed-phase liquid **chromatography** step. These each gave a single major band and several minor bands on polyacrylamide gel electrophoresis and had similar amino acid compositions. The specific activity of purified murine asialo-GM-CSF was approximately 8 x 109 colonies per mg of protein. Amino acid sequence determination of the major form gave a single amino-terminal sequence, which has been used to develop oligonucleotide probes for the isolation of two cDNA clones encoding GM-CSF. The nucleotide sequence of these two clones gave a deduced amino acid sequence almost identical with that determined for the amino terminus of asialo-GM-CSF and an amino acid composition very similar to that for asialo-GM-CSF.

L57 ANSWER 38 OF 46 EMBASE
on STN
ACCESSION NUMBER: 83205572 EMBASE
DOCUMENT NUMBER: 1983205572
TITLE: **Purification** of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor.
AUTHOR: Nicola N.A.; Metcalf D.; Matsumoto M.; Johnson G.R.
CORPORATE SOURCE: Cancer Res. Unit, Walter Eliza Hall Inst. Med. Res., Post Off. R. Melbourne Hosp., Melbourne, 3050 Vic., Australia
SOURCE: Journal of Biological Chemistry, (1983) Vol. 258, No. 14, pp. 9017-9023.
CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
ENTRY DATE: Entered STN: 911209
Last Updated on STN: 911209
ED Entered STN: 911209

Last Updated on STN: 911209

AB A naturally occurring inducer of terminal differentiation in a murine myelomonocytic leukemia cell line (WEHI-3B) was purified to apparent homogeneity from medium conditioned by lungs from mice injected with bacterial endotoxin. The factor was purified over 400,000-fold by sequential fractionation using salting out **chromatography**, **chromatography** on phenyl-Sepharose, gel filtration on Bio-Gel P-60 in 1 M acetic acid, reverse-phase high performance liquid **chromatography** on a phenyl-silica column, and high performance liquid **chromatography** on a gel filtration column. During the first two steps, the differentiation-inducing factor was separated completely from a known proliferative regulator for normal myeloid cells, granulocyte-macrophage colony-stimulating factor, but it copurified through all remaining steps with a distinct granulocyte-specific colony-stimulating factor. The purified factor showed a single protein band of M(r) = 24,000-25,000 on sodium dodecyl sulfate-polyacrylamide gels coincident with both differentiation-inducing and granulocyte colony-stimulating activity. The granulocyte-specific colony-stimulating factor was active on WEHI-3B cells and normal granulocytic progenitor cells in vitro at the same half-maximally active concentration of 3 x 10-12 M.

L57 ANSWER 39 OF 46 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2001:198128 BIOSIS
DOCUMENT NUMBER: PREV200100198128
TITLE: Expression, **purification**, and characterization of the active immunoglobulin-like domain of human granulocyte-colony-stimulating factor receptor in Escherichia coli.
AUTHOR(S): Ishibashi, Matsujiro; Tokunaga, Hiroko; Arakawa, Tsutomu; Tokunaga, Masao [Reprint author]
CORPORATE SOURCE: Laboratory of Applied and Molecular Microbiology, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima, 890-0065, Japan
tokunaga@chem.agri.kagoshima-u.ac.jp
SOURCE: Protein Expression and Purification, (March, 2001) Vol. 21, No. 2, pp. 317-322. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Apr 2001
Last Updated on STN: 18 Feb 2002

ED Entered STN: 25 Apr 2001
Last Updated on STN: 18 Feb 2002

AB We succeeded in the expression, purification, and refolding of the immunoglobulin-like (Ig) domain of human granulocyte-colony-stimulating factor (G-CSF) receptor with amino-terminal His-tag in Escherichia coli. The refolded Ig domain bound to a G-CSF affinity column and could be eluted with free G-CSF as a receptor-ligand complex, demonstrating that the Ig domain has the information necessary for binding its ligand, G-CSF. The eluted His-Ig/G-CSF complex could be separated from excess G-CSF by Ni-NTA column **chromatography**. The yield of this active recombinant His-Ig protein is about 0.72 mg per liter of culture. Its small size and the ease of production make this receptor fragment a useful reagent for the structural analysis of its complex with G-CSF.

L57 ANSWER 40 OF 46 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2001:174615 BIOSIS
DOCUMENT NUMBER: PREV200100174615
TITLE: Expression and **purification** of cytokine receptor homology domain of human **granulocyte-colony-stimulating factor** receptor fusion protein in Escherichia coli.
AUTHOR(S): Tatsuda, Daisuke; Arimura, Haruhiko; Tokunaga, Hiroko; Ishibashi, Matsujiro; Arakawa, Tsutomu; Tokunaga, Masao [Reprint author]
CORPORATE SOURCE: Laboratory of Applied and Molecular Microbiology, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima, 890-0065, Japan
tokunaga@chem.agri.kagoshima-u.ac.jp
SOURCE: Protein Expression and Purification, (February, 2001) Vol. 21, No. 1, pp. 87-91. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Apr 2001
Last Updated on STN: 18 Feb 2002
ED Entered STN: 11 Apr 2001
Last Updated on STN: 18 Feb 2002
AB Direct expression of the cytokine receptor homology (CRH) domain of granulocyte-colony-stimulating factor (G-CSF) receptor is lethal to Escherichia coli. For the efficient and stable production of an active CRH domain in E. coli, we fused the CRH domain with different proteins, such as maltose-binding protein (MalE), glutathione S-transferase, and thioredoxin (Trx). Among these, Trx appeared to be the best in terms of the protein expression level, purification efficiency by affinity **chromatography**, and binding activity to its ligand, G-CSF. The yield of active Trx-CRH fusion protein increased about 200-fold compared to that of previously reported MalE-CRH fusion.

L57 ANSWER 41 OF 46 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1992:330231 BIOSIS
DOCUMENT NUMBER: PREV199294032072; BA94:32072
TITLE: DETECTION AND **QUANTITATION** OF RECOMBINANT GRANULOCYTE COLONY-STIMULATING FACTOR CHARGE ISOFORMS COMPARATIVE ANALYSIS BY CATIONIC-EXCHANGE **CHROMATOGRAPHY** ISOELECTRIC FOCUSING GEL ELECTROPHORESIS AND PEPTIDE MAPPING.
AUTHOR(S): CLOGSTON C L [Reprint author]; HSU Y-R; BOONE T C; LU H S
CORPORATE SOURCE: AMGEN INC, AMGEN CENT, THOUSAND OAKS, CALIF 91320, USA
SOURCE: Analytical Biochemistry, (1992) Vol. 202, No. 2, pp. 375-383.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 11 Jul 1992
Last Updated on STN: 11 Jul 1992
ED Entered STN: 11 Jul 1992
Last Updated on STN: 11 Jul 1992
AB Routine quantitation of recombinant human **granulocyte colony-stimulating factor** charge isoforms in the **purified** protein product requires development of a reliable analytical method. In this report, isoelectric focusing gel electrophoresis, peptide mapping, and cation-exchange high-performance

liquid **chromatography** are compared and evaluated in the analysis of charge isomers that may be present in the recombinant factor. Due to a lack of sensitivity and reliability, isoelectric focusing gel electrophoresis and peptide mapping are not recommended. However, peptide mapping can distinguish aberrant peptides with differences in charges and provide separation for subsequent structural characterization. By this approach, an N-terminally blocked formylmethionyl species was identified to be the minor charge isoform in the **purified** preparations of recombinant human **granulocyte colony-stimulating factor**. In contrast to electrophoresis and peptide mapping, a strong cationic-exchange **chromatographic** procedure was found to be the most selective, sensitive, and reproducible analytical method. The sensitivity and reliability of the method were evaluated and validated using the formylmethionyl isoform and several deamidated analogs (Gln → Glu) made by site-directed mutagenesis. Recombinant human granulocyte colony-stimulating factor preparations contain a very low to undetectable level of the formylmethionine isoform and have no detectable deamidated isoforms.

L57 ANSWER 42 OF 46 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1988:434878 BIOSIS
DOCUMENT NUMBER: PREV198835087008; BR35:87008
TITLE: A **MICROPREPARATIVE CHROMATOGRAPHIC** STRATEGY FOR THE **PURIFICATION** AND SEQUENCE ANALYSIS OF MURINE **GRANULOCYTE-COLONY STIMULATING FACTOR.**
AUTHOR(S): NICE E C [Reprint author]; SIMPSON R J; NICOLA N A
CORPORATE SOURCE: MELBOURNE TUMOUR BIOL BRANCH, LUDWIG INST CANCER RES, AUST 3050
SOURCE: Chromatographia, (1987) Vol. 24, pp. 449-454.
Meeting Info.: ELEVENTH INTERNATIONAL SYMPOSIUM ON COLUMN LIQUID CHROMATOGRAPHY, AMSTERDAM, NETHERLANDS, JUNE 28-JULY 3, 1987. CHROMATOGRAPHIA.
CODEN: CHRGB7. ISSN: 0009-5893.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 24 Sep 1988
Last Updated on STN: 24 Sep 1988
ED Entered STN: 24 Sep 1988
Last Updated on STN: 24 Sep 1988

L57 ANSWER 43 OF 46 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1983:243147 BIOSIS
DOCUMENT NUMBER: PREV198376000639; BA76:639
TITLE: **ISOLATION** OF A HIGH MOLECULAR MASS **GRANULOCYTE COLONY STIMULATING FACTOR** FROM BOVINE LUNG CONDITIONED MEDIUM.
AUTHOR(S): NEUMEIER R [Reprint author]; MAURER H R
CORPORATE SOURCE: INST MOLEKULARBIOL BIOCHEM, FREIEN UNIV BERLIN, ARNIMALLEE 22, D-1000 BERLIN 33, W GER
SOURCE: Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie, (1982) Vol. 363, No. 12, pp. 1493-1500.
CODEN: HSZPAZ. ISSN: 0018-4888.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB A high-molecular mass type of granulocyte colony-stimulating factor (CSF-F) from bovine lung tissue produced by fibroblasts was isolated and partially purified by a 4-step procedure. Following ammonium sulfate fractionation, hydrophobic interaction, ion-exchange and affinity **chromatography**, CSF-F was > 200-fold enriched. CSF-F was a glycoprotein of .apprx. 68 kDa [kilodalton] with an isoelectric point of 4.0-5.2. It did not lose its biological activity after neuraminidase treatment. Gel-filtration behavior of desialo-CSF-F was not changed.

L57 ANSWER 44 OF 46 WPIX COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2001-184562 [19] WPIX
DOC. NO. CPI: C2001-055512
TITLE: **Purification** of **granulocyte colony-stimulating factor** comprises hydrophobic interaction **chromatography** followed by **chromatography** on **hydroxyapatite.**
DERWENT CLASS: B04
INVENTOR(S): DUMAS, J; REY, L; SARUBBI, E
PATENT ASSIGNEE(S): (AVET) AVENTIS PHARMA SA; (HMRI) HOECHST MARION ROUSSEL SA; (HMRI) HOECHST MARION ROUSSEL
COUNTRY COUNT: 86
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
FR 2796071	A1	20010112	(200119)*		29
WO 2001004154	A1	20010118	(200119)	FR	
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AU BA BB BG BR BZ CA CN CR CU CZ DM DZ EE GD GE HR HU ID IL IN IS JP KP KR LC LK LR LT LV MA MG MK MN MX NO NZ PL RO SG SI SK TR TT UA US UZ VN YU ZA					
AU 2000062940	A	20010130	(200127)		
EP 1200471	A1	20020502	(200236)	FR	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2003504378	W	20030204	(200320)		33

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
FR 2796071	A1	FR 1999-8831	19990708
WO 2001004154	A1	WO 2000-FR1937	20000706
AU 2000062940	A	AU 2000-62940	20000706
EP 1200471	A1	EP 2000-949647	20000706
		WO 2000-FR1937	20000706
JP 2003504378	W	WO 2000-FR1937	20000706
		JP 2001-509763	20000706

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000062940	A	Based on	WO 2001004154

EP 1200471 A1 Based on WO 2001004154
JP 2003504378 W Based on WO 2001004154

PRIORITY APPLN. INFO: FR 1999-8831 19990708
AB FR 2796071 A UPAB: 20010405
NOVELTY - Process for **purifying granulocyte colony-stimulating factor** (**G-CSF**) from a biological sample comprises:
(a) reducing the volume of the sample by hydrophobic interaction **chromatography** to obtain a concentrated, desalted and enriched fraction (F1);
(b) passing F1 over **hydroxyapatite** under conditions such that **G-CSF** is weakly bound to obtain **concentrated**, desalted and enriched fraction (F2) containing **G-CSF**; and
(c) recovering **G-CSF** from F2.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a process for removing contaminating proteins from a **G-CSF** solution, comprising passing the solution over **hydroxyapatite** so that the contaminating proteins are bound to the **hydroxyapatite** while **G-CSF** is weakly bound, and recovering the **G-CSF**.
USE - the method is useful for **purifying granulocyte colony-stimulating factor** (**G-CSF**) from a biological sample.
ADVANTAGE - **G-CSF** with a purity of at least 90% can be obtained.
Dwg.0/7
ABEX UPTX: 20010405
EXAMPLE - Culture supernatant from a human cell line expressing gene activation G-CSF (GA-GCSF) was adjusted to 0.5 M NaCl, filtered, and loaded (1640 ml) onto a column of Phenyl Sepharose (RTM) Fast Flow High Substitution (50 ml). The column was washed with 0.5 M NaCl and eluted with demineralized water in 2 ml fractions. Pooled active fractions (50 ml) contained 45.1 mg GA-GCSF. The product (24 ml) was loaded onto a column of hydroxyapatite equilibrated with 1 mM phosphate buffer (pH 7.3). The column was eluted with 1 mM phosphate buffer (pH 7.3) in 1 ml fractions. Pooled active fractions (25 ml) contained 21.3 mg GA-GCSF with a purity of 97.8%, corresponding to a yield of 98.4%.

L57 ANSWER 45 OF 46 WPIX COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2000-397851 [34] WPIX
DOC. NO. CPI: C2000-119835
TITLE: Method for **purification** of human **granulocyte colony stimulating factor** by high performance liquid **chromatography** (HPLC) - NoAbstract.
DERWENT CLASS: B04 D16
INVENTOR(S): HA, S H; HAM, S H; KIM, H S; KIM, K W; KOH, H K; LEE, H S; MOK, K H; CHUNG, G T; CHUNG, J S; HAH, S H; KIM, G W; KOH, H G; MOK, H
PATENT ASSIGNEE(S): (CHEI-N) CHEIL JEDANG CO; (CHEI-N) CHEIL FOODS & CHEM INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
KR 99040733	A	19990605	(200034)*		
KR 266541	B1	20000915	(200134)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
KR 99040733	A	KR 1997-61204	19971119
KR 266541	B1	KR 1997-61204	19971119

PRIORITY APPLN. INFO: KR 1997-61204 19971119

L57 ANSWER 46 OF 46 WPIX COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 1988-247031 [35] WPIX
DOC. NO. NON-CPI: N1988-187839
DOC. NO. CPI: C1988-110670
TITLE: Anti-G-CSF mono clonal antibody - to human **granulocyte colony stimulation factor**, used for **purificn.** and determn. of **G-CSF.**
DERWENT CLASS: B04 D16 S03
PATENT ASSIGNEE(S): (KYOW) KYOWA HAKKO KOGYO KK
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 63180860	A	19880725	(198835)*		7

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 63180860	A	JP 1987-12721	19870122

PRIORITY APPLN. INFO: JP 1987-12721 19870122
AB JP 63180860 A UPAB: 19930923
Anti-G-CSF monoclonal antibody to human granulocyte colony stimulation factor (G-CSF) is claimed.

Specifically the monoclonal antibody has neutralising activity to G-CSF activity. Spleen cell obtd. by immunising a mouse with G-CSF is fused with the myeloma cell of a mouse. The obtd. hybridoma is cultured in a medium or transplanted into the peritoneal cavity of a mouse to form and accumulate anti-G-CSF monoclonal antibody in the cultured liquid or the peritoneal juice, and the monoclonal antibody is separated from the cultured liquid or peritoneal juice. The **purificn.** of **G-CSF** is carried out by **isolating G-CSF** from a substance containing **G-CSF** by affinity **chromatography** using the anti-G-CSF monoclonal antibody. The determn. of G-CSF is carried out by enzyme immunoassay by solid phase sandwich method using the anti-**G-CSF** monoclonal antibody. For the **purificn.** of anti-**G-CSF** antibody, it is passed through DEAE-cepharose column, etc. and IgG fraction is collected.

USE/ADVANTAGE - Useful for the **purificn.** and determn. of **G-CSF. G-CSF** is efficiently **purified** and determined simply and rapidly with high sensitivity using the anti-G-CSF monoclonal antibody.
0/2

FILE 'HOME' ENTERED AT 12:15:58 ON 06 MÀY 2005

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=> d his

     (FILE 'HOME' ENTERED AT 10:05:47 ON 06 MAY 2005)

     FILE 'STNGUIDE' ENTERED AT 10:06:01 ON 06 MAY 2005

     FILE 'HOME' ENTERED AT 10:06:06 ON 06 MAY 2005

     FILE 'ZCAPLUS' ENTERED AT 10:07:20 ON 06 MAY 2005
               E HYDROXYAPATITE/CT
               E E3
               E E3+ALL
               E E2+ALL

     FILE 'ZREGISTRY' ENTERED AT 10:13:27 ON 06 MAY 2005

     FILE 'REGISTRY' ENTERED AT 10:52:38 ON 06 MAY 2005
               E 143011-72-7
L1             1 S E3
               E 1306-06-5
L2             1 S E3

     FILE 'ZCAPLUS' ENTERED AT 10:54:06 ON 06 MAY 2005

     FILE 'CAPLUS' ENTERED AT 11:07:49 ON 06 MAY 2005
L3          9750 S L1 OR GRANUL? COLON? STIMUL? FACTOR OR G CSF OR GCSF OR RM DF
L4         18620 S L2 OR HYDROXYLAPATITE+PFT/CT OR APP 600 OR AMDRY 6021 OR HA 2
L5        173049 S CHROMATOGRAPHY/CW
L6             1 S L3 AND L4 AND L5
L7            59 S L3 AND L5
L8            44 S L1 (L) PUR/RL
L9            22 S L7 AND L8
L10          356 S L1 (L) PREP/RL
L11           24 S L7 AND L10
L12           24 S L9 OR L11

     FILE 'MEDLINE' ENTERED AT 11:17:38 ON 06 MAY 2005
L13        12827 S L3
L14         6783 S L4
L15       400631 S CHROMATOGRAPH?
L16            1 S L13 AND L14 AND L15
L17          165 S L13 AND L15
L18         7584 S GRANULOCYTE COLONY-STIMULATING FACTOR/CT
L19         4112 S L18/MAJ
L20           19 S L19 (L) IP/CT
L21           11 S L20 AND L15
L22            6 S L21 NOT (PICHIA OR STROMAL OR DIMER? OR LIGAND OR GLYCOL)/TI

     FILE 'EMBASE' ENTERED AT 11:26:09 ON 06 MAY 2005
L23        20200 S L3
L24         6967 S L4
L25       299366 S CHROMATOGRAPH?
L26            0 S L23 AND L24 AND L25
L27          178 S L23 AND L25
L28        13008 S GRANULOCYTE COLONY STIMULATING FACTOR/CT
L29           73 S L28 AND L25
L30         4733 S L28/MAJ
L31           30 S L30 AND L25
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L32             8 S L31 AND (ISOLATION OR PURIF? OR RETENTION)/TI

     FILE 'BIOSIS' ENTERED AT 11:35:02 ON 06 MAY 2005
L33         15668 S L3
L34          6240 S L4
L35        384841 S CHROMATOG?
L36             1 S L33 AND L34 AND L35
L37           136 S L33 AND L35
L38       2073071 S ISOLAT? OR PURIF? OR CONCENT?
L39            94 S L33 AND L35 AND L38
L40            42 S L33 (10A) L38 AND L35
L41            40 S L33 (8A) L38 AND L35
L42            10 S L41 AND (PURIFICATION OR QUANTITATION OR MICROPREP? OR ISOLAT

     FILE 'WPIX' ENTERED AT 11:44:50 ON 06 MAY 2005
L43          1222 S GRANUL? COLON? STIMUL? FACTOR OR G CSF OR GCSF OR RM DF OR GR
L44          3463 S HYDROXYLAPATITE OR HYDROXYAPATITE OR APP 600 OR AMDRY 6021 OR
L45         45669 S CHROMATOGRA?
L46             2 S L43 AND L44 AND L45
L47             1 S L46 NOT HERPES/TI
L48            12 S L43 (10A) (ISOLAT? OR PURIF? OR CONC?) AND L45
L49             7 S L48 NOT (HAEMATO? OR MASTITIS OR SUPERGENE OR SULFATE OR MONO

     FILE 'CAPLUS' ENTERED AT 11:57:23 ON 06 MAY 2005

     FILE 'MEDLINE' ENTERED AT 11:57:33 ON 06 MAY 2005
L50             7 S L16 OR L22

     FILE 'EMBASE' ENTERED AT 11:58:02 ON 06 MAY 2005

     FILE 'BIOSIS' ENTERED AT 11:58:21 ON 06 MAY 2005
L51            11 S L36 OR L42

     FILE 'WPIX' ENTERED AT 11:59:05 ON 06 MAY 2005
L52             7 S L47 OR L49

     FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS, WPIX' ENTERED AT 12:00:04 ON 06
     MAY 2005
L53            46 DUP REM L50 L12 L32 L51 L52 (11 DUPLICATES REMOVED)

     FILE 'HOME' ENTERED AT 12:01:12 ON 06 MAY 2005

     FILE 'CAPLUS' ENTERED AT 12:03:58 ON 06 MAY 2005

     FILE 'REGISTRY' ENTERED AT 12:09:54 ON 06 MAY 2005

     FILE 'REGISTRY' ENTERED AT 12:10:23 ON 06 MAY 2005

     FILE 'CAPLUS' ENTERED AT 12:11:42 ON 06 MAY 2005

     FILE 'MEDLINE' ENTERED AT 12:11:52 ON 06 MAY 2005

     FILE 'EMBASE' ENTERED AT 12:12:09 ON 06 MAY 2005

     FILE 'MEDLINE' ENTERED AT 12:12:36 ON 06 MAY 2005
L54             7 S L16 OR L22

     FILE 'BIOSIS' ENTERED AT 12:13:11 ON 06 MAY 2005
L55            11 S L36 OR L42
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     FILE 'WPIX' ENTERED AT 12:13:55 ON 06 MAY 2005
L56             7 S L47 OR L49

     FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS, WPIX' ENTERED AT 12:15:09 ON 06
     MAY 2005
L57            46 DUP REM L54 L12 L32 L55 L56 (11 DUPLICATES REMOVED)

     FILE 'HOME' ENTERED AT 12:15:58 ON 06 MAY 2005

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